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A comparison of virus isolation, immunohistochemistry, fetal serology, and reverse-transcription polymerase chain reaction assay for the identification of porcine reproductive and respiratory syndrome virus transplacental infection in the fetus

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Abstract. Virus isolation (VI), immunohistochemistry (IHC), fetal serology, and reverse-transcription polymerase chain reaction assay (RT-PCR) were performed on samples from 107 fetuses comprising 10 litters taken from sows experimentally infected with porcine reproductive and respiratory syndrome virus (PRRSV). In addition to comparing the relative sensitivity and specificity of each test, RT-PCR was evaluated with respect to the relative suitability of thoracic fluids and tissues as samples, the effects of autolysis, and the effects of pooling of fetal specimens. VI, IHC, and fetal serology identified PRRSV infection in 48.6%, 23.4%, and 14.9% of 107 fetuses, respectively, and identified at least 1 infected fetus in 10, 10, and 5 of 10 litters, respectively. In utero death with autolysis reduced the test efficacy of all 3 methods. Fetal thoracic fluid and tissues were equally suitable for RT-PCR detection of PRRSV. Pooling fetal tissues or fluids from VI-positive animals with comparable material from negative controls had no detrimental effect on RT-PCR results when evaluated at dilutions of 1:1, 1:2, 1:4, and 1:8. The results of RT-PCR testing were positive in 100%, 94.4%, and 83.3% of VI-positive specimens allowed to autolyze at 4, 21, or 37 C, respectively, for 24, 48, and 96 hours. Compared with the other testing modalities, RT-PCR appeared to be impacted the least by the adverse effects of autolysis.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major cause of reproductive failure in swine. The disease is characterized by late-term abortion and the birth of a mixture of weak and stillborn pigs and dead, autolyzed fetuses.[17] Porcine reproductive and respiratory syndrome (PRRS) can be difficult to diagnose because fetuses are the most common specimen submitted to diagnostic laboratories in abortion cases, and there are no consistent gross or microscopic lesions of this syndrome in fetuses.[17] Development of practical techniques for assessing the PRRSV status of fetuses would greatly improve the ability of diagnosticians to identify PRRSV-induced abortion and allow producers and veterinarians to more efficiently manage reproductive losses.

Although PRRSV can be readily isolated from the serum or tissues of presuckled, congenitally infected piglets, in utero autolysis rapidly inactivates the virus and interferes with isolation from aborted transplacentally infected fetuses.[12,21] Consequently, virus isolation (VI) for PRRSV from fetuses submitted from field cases of abortion has been extremely disappointing. Other potential techniques for the diagnosis of transplacental PRRSV infection include immunohistochemistry (IHC), fetal serology, and the reverse-transcription polymerase chain reaction assay (RT-PCR). Immunohistochemical techniques have been used extensively to identify PRRSV antigen in numerous tissues.[9,10] Fetal serology has been examined in a variety of abortion diseases, albeit with mixed results.[1,3,8,11,14,18,19,22] Because PRRSV infection typically causes late gestation reproductive failure, there is the potential for aborted or stillborn fetuses to develop a detectable immune response. RT-PCR has been used for the detection of PRRSV nucleic acids in serum and tissues.[6,7,20] Because successful application of RT-PCR techniques relies on the presence of genetic material rather than infectious virus, RT-PCR can be used to detect inactivated or incomplete virions. In addition, the amplification properties of RT-PCR allow detection of very low concentrations of the target material.

In this study, VI, fetal serology, IHC, and RT-PCR techniques were compared for their ability to detect transplacental PRRSV infection in fetuses derived from sows experimentally infected with PRRSV during late gestation. In addition, the effect on RT-PCR of fetal autolysis and pooling of tissues and serum or thoracic fluids from multiple fetuses and the relative suitability of thoracic fluids and tissues for RT-PCR were evaluated.

Materials and methods

Experimental animals

As part of a related study,[2] naturally mated pregnant sows were obtained from a herd deemed free of PRRSV based on clinical and serologic history. Sows were seronegative for PRRSV at purchase and following 2 wk in isolation. Ten randomly selected animals were exposed to PRRSV on or about day 90 of gestation by intramuscular injection of PRRSV strain NADC-8. Similar sows challenged with a sham inoculum served as controls. All animals were eutha-

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nized at 21 days postinoculation (DPI) (approximately day 111 of gestation) and necropsied. Serum was collected to assess for seroconversion in exposed animals. Fetuses were collected from each sow at necropsy. Fetuses were deemed live if they had an umbilical pulse or heartbeat. Fetuses identified as dead included autolyzed fetuses and fetuses that appeared fresh but lacked a heartbeat or umbilical pulse. Serum or thoracic fluid from live and dead fetuses, respectively, and tissues (brain, lung, cardiac muscle, liver, spleen, tonsil, placenta, umbilical cord, aorta, thymus, and mediastinal lymph nodes) were collected and held at −70 C pending testing. Similar tissues were routinely fixed in 10% neutral buffered formalin. Fetal specimens from sham-challenged sows served as laboratory test controls.

Challenge virus

The NADC-8 PRRSV strain was prepared as previously described.[14] The virus was isolated from serum of a weak-born pig on MARC-145 cells. The cell culture was frozen and thawed, and the virus was passed 2 more times. Third-passage virus was titrated and diluted with serum-free minimal essential medium (MEM) to prepare challenge virus inoculum. A virus-free control sham inoculum was prepared in a similar fashion from MARC-145 cells.

Virus isolation

Virus isolation with fetal serum or thoracic fluid was performed as previously described.[13–15] Cultured cells of the MARC-145 cell line were propagated in Eagle MEM supplemented with 10% fetal calf serum and gentamycin sulfate (0.05 mg/ml). Two hundred microliters of the appropriate sample was added to the nutrient medium (1 ml) of a confluent monolayer of MARC-145 cells and incubated at 37 C in a humid atmosphere of 5% CO_2. Cell cultures were examined daily for 7 days for cytopathic effect. Culture medium (0.2 ml) from the inoculated wells was used to inoculate a second passage when primary isolation was unsuccessful. Lack of cytopathic effect in these cultures was interpreted as a negative test.

Fetal serology

Samples of fetal serum or thoracic fluid from live or dead fetuses, respectively, were tested for PRRSV antibodies by indirect fluorescent antibody (IFA) test as previously described.[23] MARC-145 cells were propagated and seeded onto 8-chamber slides. Cell cultures were incubated in Eagle MEM with 10% fetal bovine serum plus 0.25 μg/ml amphotericin-B and 50 μg/ml gentamicin in a humidified chamber at 37 C and with 5% CO_2 until 70–80% monolayering was acheived (about 34 hr). Strain NADC-8 PRRSV-infected culture medium containing sufficient virus to produce 15–20 plaque-forming units was added to each chamber, and the cultures were incubated for 24–36 hr. Culture medium was removed, and the monolayers were fixed in 80% aqueous acetone for 10 min and stored at −80 C until used. Serum samples were diluted 1:20 in phosphate-buffered saline (PBS), 50 μl of diluted serum was placed in each chamber, and the slides were incubated for 30–45 min at 37 C. Slides were washed with successive changes of PBS followed by distilled water, were coated with 50 μl goat anti-swine IgG conjugated with fluorescein isothiocyanate, were incubated 30–45 min, and were examined by fluorescent microscopy. Test monolayers were compared with negative and positive controls; positive status was based on the presence of typical foci of fluorescence.

Immunohistochemistry

Immunohistochemical staining of fetal lung, thymus, liver, spleen, and umbilical cord was performed as previously described[9] using a modified automated procedure. Tissues were fixed in 10% neutral buffered formalin and routinely processed in an automated tissue processor to produce paraffin blocks. Three-micrometer-thick sections were mounted on poly-L-lysine–coated glass slides, deparaffinized with 2 changes of xylene, and rehydrated through graded alchohol baths to distilled water. Endogenous peroxidase was removed by 3% hydrogen peroxide and digestion with 0.05% protease. Slides were prepared in an automated immunohistochemical processor utilizing primary monoclonal antibody ascites fluids containing antibodies SDOW-17[a] and SR-30[a] diluted 1:1,000 in Tris-PBS followed by biotinylated goat anti-mouse linking antibody, peroxidase-conjugated streptavidin, and 3,3′-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin.

RT-PCR

Individual fetuses. Pooled tissue samples (lung, liver, brain, spleen, umbilical cord) from each fetus were placed in a separate sterile whirl-pak bag. Two milliliters of sterile Hanks buffer per gram of fetal tissue composite was added, and the tissues were homogenized for 120 sec in a stomacher. One milliliter of the resulting homogenate was drawn off with a separate sterile pipet and held at −70 C until tested by RT-PCR.

Effect of pooling. Tissue homogenate and thoracic fluid from each of 6 VI/RT-PCR–positive fetuses were pooled with similarly processed tissue homogenate or thoracic fluid from VI/RT-PCR–negative control fetuses at dilution ratios of 1:1, 1:2, 1:4, and 1:8, and the resulting pooled specimens were tested by RT-PCR.

Effect of fetal autolysis. Pooled tissues (brain, liver, spleen, lung, umbilical cord) from 9 PRRSV VI-positive fetuses were divided evenly into 4 aliquots on individual weighing boats with a separate sterile scalpel blade under a laminar flow hood, placed in separate whirl-pak bags, and incubated for 0, 24, 48, or 96 hr to mimic postmortem decomposition. Groups A, B, and C, each consisting of samples from 3 fetuses, were stored at 4 C, 21 C, and 37 C, respectively. After incubation, 1 ml of free tissue fluid was drawn off each sample with a separate sterile pipet to mimic thoracic fluid. Gloves were changed between samples. Samples incubated at 37 C were processed first because these samples were the most likely to be negative. Two milliliters of sterile Hanks buffer per gram of fetal tissue was added to the remaining tissue, and the tissues were homogenized for 120 sec. One milliliter of tissue homogenate from each sample was drawn off with a separate sterile pipet. Each of the resulting 63 tissue homogenate and thoracic fluid samples was held at −70 C until tested by RT-PCR.

Comparison of tissue and thoracic fluid RT-PCR. Tho-

racic fluid from 20 fetuses whose tissue homogenates were positive by VI/RT-PCR and from 9 fetuses whose tissue homogenates were negative by VI/RT-PCR were assessed, and the tissue and fluid results were compared.

RT-PCR procedure. Samples of tissue and fetal fluids were analyzed by RT-PCR as previously described.[5,6] Sterile gloves were worn routinely throughout the procedures and repeatedly changed as appropriate. Tissue samples were homogenized in 2 ml Hanks balanced salt solution per gram of tissue. Five hundred microliters of serum, thoracic fluid, or tissue homogenate was added to an equal volume of lysis buffer consisting of 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. Five hundred microliters of the lysed product was then added to an equal volume of phenol chloroform–isoamyl alchohol, vortexed, and centrifuged at 10,000 × *g* for 5 min. Extraction with phenol chloroform–isoamyl alchohol was repeated, and the upper phase was transferred to 500 μl chloroform–isoamyl alchohol and centrifuged. One-third volume of 2 M sodium acetate (pH 4) and 2 volumes of cold 95% ethanol were added to the sample, and the sample was frozen at −70 C for 1 hr. The sample was centrifuged at 16,000 × *g*, washed twice in 70% ethanol, resuspended in 30 μl sterile distilled water,[b] and held at −70 C.

Outer and nested primers were derived from open reading frame 7 of the PRRSV strain VR-2332 genome. The outer sense and antisense primers included nucleotides 2763–2785 (5′-TCGTGTTGGGTGGCAGAAAAGC-3′) and nucleotides 3247–3225 (5′-GCCATTCACCACACATTCTTCC-3′), respectively. The nested sense and antisense primers included nucleotides 2885–2907 (5′-CCAGATGCTGGGTAAGATCATC-3′) and nucleotides 3121–3099 (5′-CAGTGTAACTTATCCTCCTGA-3′), respectively.

A commercially available RT-PCR system[c] was utilized for reverse transcription and outer and nested RT-PCR reactions. The outer and nested reactions consisted of 40 and 30 cycles, respectively. Denaturing, annealing, and extension temperatures and times were 95 C for 25 sec, 58 C for 5 sec, and 74 C for 25 sec, respectively. A 484-base pair (bp) outer and 236-bp nested product were visualized on a 1% agarose gel[d] containig 0.5 μg ethidium bromide/ml of agarose. The gel was subsequently photographed under ultraviolet illumination. This RT-PCR assay could detect as few as 10 virions/ml.[6]

Results

All exposed females seroconverted by 21 DPI. A total of 107 fetuses from 10 litters were evaluated for evidence of PRRSV infection by VI, IHC, and fetal serology. Tissues from 94 of these fetuses were also evaluated by RT-PCR. Of the 107 total, 34 (31.8%) were dead and 73 (68.2%) were deemed alive at the time of necropsy at 21 DPI. Results are tabulated in Table 1 (data from fetuses negative by VI, IHC, and fetal serology not shown). Specimens from 50 fetuses derived from 4 control litters were negative by all methods.

Virus isolation

Fifty-two of 107 fetuses (48.6%) were positive by VI, including 4 of 34 dead fetuses (11.8%) and 48 of 73 live fetuses (65.7%). Of the 52 VI-positive fetuses, 48 (92.3%) were deemed alive at the time of sow necropsy. At least 1 VI-positive fetus was identified in each litter.

Immunohistochemistry

Twenty-five of 107 fetuses (23.4%) were positive by IHC. Thymus, liver, spleen, lung, and umbilical cord were positive in 25, 16, 15, 8, and 5 specimens, respectively. Thymus was the only tissue to be positive in all IHC-positive fetuses. All of the 25 IHC-positive fetuses were identified as positive by VI. Twenty-four of 25 IHC-positive fetuses (96%) were deemed alive at the time of sow necropsy. All litters had at least 1 IHC-positive fetus. The sensitivity of IHC compared with VI was 48.1% and 100% on a per fetus and per litter basis, respectively, when all 5 tissues were assessed. Specificity compared with VI was 100%.

Fetal serology

Sixteen of 107 (14.9%) fetuses were positive by fetal serology. All 16 were positive by VI, and only 3 were positive by IHC. Fifteen of 16 (93.8%) were alive at sow necropsy. Five of 10 (50%) litters had at least 1 serologically positive fetus. The sensitivity of fetal serology compared with VI was 30.8% and 50% on a per fetus and per litter basis, respectively. Specificity compared with VI was 100%.

RT-PCR

Individual samples. Of the 94 samples evaluated, 88 (93.6%) were RT-PCR positive. Forty-seven samples were VI/RT-PCR positive, 4 samples were VI positive/RT-PCR negative, 41 samples were RT-PCR positive/VI negative, and 2 samples were VI/RT-PCR negative. Of the samples that were RT-PCR positive and VI negative, 28 (68.3%) came from pigs that were dead in utero.

Pooled samples. Forty-eight samples were tested, representing 6 VI/RT-PCR–positive tissue homogenates and 6 VI/RT-PCR–positive thoracic fluids, each pooled at 1:1, 1:2, 1:4, and 1:8 with similar specimens from negative control animals that were VI/RT-PCR negative. All 48 pooled samples were positive by RT-PCR.

Autolyzed samples. All tissue and thoracic fluid samples from VI-positive fetuses were RT-PCR positive at time 0 and at each postincubation time period, with the exception of 2 tissue samples (21 C/48 hours and 37 C/48 hours) and 2 thoracic fluid samples (37 C/24 and 48 hours) (Table 2). Of 54 incubated samples, 50 (92.6%) were positive. Eighteen of 18 (100%)

Table 1. Results of virus isolation (VI), immunohistochemistry (IHC), fetal serology, and reverse-transcription polymerase chain reaction (RT-PCR) testing of fetuses from sows infected with porcine reproductive and respiratory syndrome virus. Fetuses with all negative values for VI, IHC, and serology not shown.

Sow no.	Pig no.	Live/dead	VI	IHC (positive tissue*)	Serology	RT-PCR
10	1	live	pos	thy	neg	pos
	2	live	pos	sp, thy, li	neg	pos
	3	live	pos	neg	neg	neg
	4	live	pos	neg	neg	pos
	6	live	pos	neg	neg	pos
11	12	live	pos	spl, thy, li, lu	neg	not tested
12	2	live	pos	spl, thy, li	neg	pos
	5	live	pos	spl, thy	neg	pos
	6	live	pos	neg	pos	pos
	7	live	pos	neg	pos	pos
	8	live	pos	neg	pos	pos
	9	live	pos	neg	pos	pos
	10	live	pos	neg	pos	pos
14	1	live	pos	spl, thy, li, lu	neg	pos
	2	live	pos	spl, thy, li, lu	neg	pos
	3	dead	pos	spl, thy, li, lu umb	neg	pos
	5	live	pos	spl, thy, li, lu	neg	pos
	7	live	pos	neg	neg	neg
	8	live	pos	neg	neg	pos
	9	live	pos	neg	neg	pos
15	1	live	pos	neg	neg	neg
	4	dead	pos	neg	pos	pos
	7	live	pos	neg	pos	pos
	8	live	pos	neg	pos	pos
	9	live	pos	neg	pos	pos
	10	live	pos	thy, lu, umb	pos	pos
16	1	live	pos	thy	neg	pos
	2	live	pos	spl, thy, li, umb	pos	pos
	3	live	pos	neg	pos	pos
	4	live	pos	thy, li	neg	pos
	5	live	pos	spl, thy, li, lu	neg	pos
	6	live	pos	spl, thy, li, lu, umb	neg	pos
	7	live	pos	neg	neg	pos
17	2	live	pos	thy, li	neg	pos
	3	dead	pos	neg	neg	pos
	4	live	pos	thy, li	neg	pos
18	2	live	pos	spl, thy, li, umb	pos	pos
	3	live	pos	spl, thy, li	neg	pos
	4	dead	pos	neg	neg	pos
	8	live	pos	thy	neg	pos
	10	live	pos	neg	pos	pos
	11	live	pos	neg	pos	pos
	12	live	pos	thy	neg	pos
19	8	live	pos	neg	pos	neg
	10	live	pos	thy	neg	pos
	12	live	pos	spl, thy	neg	pos
	13	live	pos	neg	neg	pos
20	1	live	pos	neg	neg	pos
	2	live	pos	neg	neg	pos
	5	live	pos	thy	neg	pos
	8	live	pos	neg	neg	pos
	9	live	pos	spl, thy, li	neg	pos

* thy = thymus; li = liver; lu = lung; spl = spleen; umb = umbilical cord.

Table 2. Results of reverse-transcription polymerase chain reaction (RT-PCR) analysis of fetal pig tissue and thoracic fluid positive for porcine reproductive and respiratory syndrome virus following autolysis at various temperatures.

RT-PCR sample	Temp (°C)	Tissue			Fluid		
		24 hr	48 hr	96 hr	24 hr	48 hr	96 hr
A	4	pos	pos	pos	pos	pos	pos
B	4	pos	pos	pos	pos	pos	pos
C	4	pos	pos	pos	pos	pos	pos
D	21	pos	neg	pos	pos	pos	pos
E	21	pos	pos	pos	pos	pos	pos
F	21	pos	pos	pos	pos	pos	pos
G	37	pos	neg	pos	pos	pos	pos
H	37	pos	pos	pos	neg	pos	pos
I	37	pos	pos	pos	pos	pos	neg

samples held at typical refrigerator temperature (4 C, group A), 17 of 18 (94.4%) at room temperature (21 C, group B), and 15 of 18 (83.3%) at normal body temperature (37 C, group C) remained positive (Table 2).

Comparison of tissue and thoracic fluid RT-PCR. All 20 thoracic fluid samples from fetuses whose tissue homogenates were positive by VI/RT-PCR were also RT-PCR positive. All 9 thoracic fluid samples from fetuses whose tissue homogenates were negative by VI/RT-PCR were negative. If the 27 pairs of samples from the autolysis study are included, RT-PCR results for tissue and thoracic fluid were in agreement in 43 of the 47 (91%) sets of samples. Two samples in the autolysis study tissue pools were positive while the thoracic fluid was negative, and 2 samples of thoracic fluid were positive while the tissue pools were negative.

Discussion

The sensitivity and specificity of diagnostic tests are generally based on a ''gold standard.'' Unfortunately, there is currently no recognized gold standard for the detection of PRRSV in transplacentally infected fetuses. In live pigs, swine bioassay may be the most sensitive test for the detection of PRRSV. However, this standard may not be applicable to aborted fetuses because of the complications of in utero death and fetal autolysis. In the present study, VI was used as the standard against which the other tests were evaluated.

It was predicted that fetal serology would detect a large percentage of PRRSV-infected fetuses because sows were inoculated when fetuses were immunocompetent and the majority of fetuses were collected at 21 DPI. Antibody has been demonstrated in fetal serum under similar experimental circumstances.[14] The IFA test was chosen in this trial to allow use of the challenge virus as the cell culture inoculum. The IFA tests to detect IgG specific for PRRSV antigen were positive in only 14.9% of the fetuses. The IFA tests were quite specific; titers were detected only in VI-positive fetuses. However, fetal serology detected only 16 (30.8%) of 52 VI-positive fetuses, and 1 or more IFA-positive fetuses were detected in only 5 of 10 (50%) infected litters. Even if the entire litter is evaluated, fetal serology appears to be of limited value. In this experimental model, fetal samples were collected at 21 DPI; in field cases utilizing full-term weakborn, presuckled piglets, the duration of intrauterine exposure may be longer, resulting in a higher percentage of positive animals. Application of serologic testing could be enhanced by the use of an IgM-rather than an IgG-based test because IgM levels rise before IgG levels.

Immunohistochemistry was more sensitive than fetal serology under the conditions of this study. When thymus, liver, spleen, lung, and umbilicus from each fetus were assessed on a single IHC slide, PRRSV antigen was detected in 1 or more tissues of 25 fetuses. Overall, IHC detected PRRSV in 23.4% of fetuses and 48.1% of VI-positive animals. IHC detected PRRSV antigen in at least 1 fetus in all VI-positive litters. In fetuses in which PRRSV antigen was detected in 1 or more tissues, positive cells were identified in 100% of the thymuses, 64% of the livers, 60% of the spleens, 32% of the lungs, and 20% of the umbilical cords. These results indicate that tissue selection may have a dramatic impact on the sensitivity of PRRSV IHC. However, these results might not be relevant to all strains of PRRSV. In a report involving a limited number (13) of naturally infected fetuses, IHC demonstrated PRRSV in fetal spleen, lung and liver, and thymus in 7, 5, and 4 fetuses, respectively.[4]

The results of this study emphasize the potential impact of autolysis on the detection of PRRSV in fetuses. The adverse effect of autolysis on VI results has been well documented.[12,21] Of the 107 fetuses evaluated, 34 (31.8%) were dead and 73 (68.2%) were alive at the time of sow necropsy. Four of the dead fetuses (11.8%) were VI positive, and 48 of the live fetuses (65.7%) were VI positive. Of the 52 VI-positive fe-

tuses, 48 (92.3%) were alive at the time of necropsy at 21 DPI. These results are in agreement with those of other studies in which VI rates in PRRSV-positive tissues decreased to 47% and 7% after 24 and 72 hours of storage at 25 C, respectively.[21] Similarly stored serum was less affected in that study.

Fifteen of 16 (93.8%) serologically positive fetuses were alive at the time of sow necropsy. Autolysis has been observed to negatively influence fetal serology in other abortion-related diseases.[16] However, considering the dynamics of fetal infection and immune response, there is a strong possibility that the dead fetuses died prior to the development of an immune response.

Perhaps the most intriguing finding of this study is the limited impact of autolysis on PRRSV RT-PCR. Autolysis had a limited impact even at in utero temperatures and had minimal impact under typical refrigeration and room temperatures. The decrease in sensitivity of RT-PCR under conditions of autolysis compares favorably to the more severe decrease in VI rates under similar conditions.[21] Thus, RT-PCR should be a valuable diagnostic tool for evaluating the autolyzed specimens routinely submitted to diagnostic laboratories.

Pooling of 1 positive tissue or thoracic fluid sample with up to 8 negative similar samples did not influence the ability to detect PRRSV with RT-PCR. This magnitude of dilution mimics that which would result from pooling specimens from all fetuses within a litter. In addition, both tissue and thoracic fluids gave similar results when tested by RT-PCR. These results suggest that RT-PCR testing of carefully collected field samples of thoracic fluid or fetal serum from presuckled piglets or stillborn fetuses would be a practical approach to diagnosis. Pooling of such samples from multiple fetuses will decrease the possibility of missing infected specimens and will reduce laboratory cost and labor.

A high percentage of individual fetuses were positive by RT-PCR. Three possibilities should be considered for these results. All fetuses were from sows challenged with PRRSV; thus, all RT-PCR–positive fetuses may have been infected with the virus. This study demonstrated the negative impact of autolysis on VI and the limited impact of autolysis on RT-PCR. Sixty-eight percent of the fetuses that were VI negative and RT-PCR positive were dead in utero. These findings suggest that in autolyzed specimens, RT-PCR was indeed more sensitive than VI. Similar findings have been reported for naturally infected fetuses.[4] However, another explanation for the high numbers of RT-PCR–positive fetuses may be cross-contamination between fetuses at postmortem. It appears that the procedures to minimize cross-contamination were sufficient between sows, because all samples from negative control animals remained negative. But considering the sensitivity of RT-PCR and the facilities available for necropsy, the possibility of cross-contamination of fetuses within a litter does exist, and thus the results of this study should be interpreted with caution.

Sources and manufacturers

a. Rural Technologies, Brookings, SD.
b. Gibco BRL, Grand Island, NY.
c. GeneAmp RT/PCR kit, Applied Biosystems, Foster City, CA.
d. SeaKem, FMC Bioproducts, Rockland, MA.

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